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Microorganisms contaminated in the process of cultivation and their effect on the production of shiitake[1]

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Abstract : Contamination has been a major problem for the shiitake production in Taiwan. To eliminate the contamination to a level as low as possible is very important. In this study, the contaminated microorganisms were identified and the sources of contaminants in the sawdust, spawn, inoculation and incubation rooms, and cultivation yard were described. Attempts were also made to explore the effects of the contaminants on the mycelial growth and/or the yield of shiitake. The effect of medium pH values on the mycelial growth of shiitake and contaminants were also reported. Results indicated that species of *Trichoderma, Penicillium, Neurospora* and *Aspergillus* were the most frequently isolated fungi from the sawdust spawn, while *Trichoderma, Bacillus* and *Aspergillus* were often isolated in the wheat grain spawn. The mycelium of shiitake grew more quickly in the wheat grain medium than it did in the sawdust medium. More microorganisms were trapped in the cultivation yard than in the incubation room. Unused sawdust piled outdoor for 6 months contained more microorganisms than the cultivating one. During marketing or transporting process, population of microorganisms on the surfaces of shiitake fruiting bodies increased greatly. Less amount of microorganisms were found in the normally grown sawdust than in the contaminated sawdusts. The pH value of contaminated sawdust was higher than the normal one, but not much difference was observed in the water contents of the two kind of sawdusts. The pH value of *Castanopsis hytrix* saw-dust was very low within 2 months of piling, but it gradually increased with time and reached ca. 6.0 within 3-4 months which will be suitable for medium perparation. The pH values of *Acacia confusa* and broad leaf trees also increased during piling. The mycelia of shiitake grew best in the acidic conditions when the pH value was between 4.5 and 6.0, while *Trichoderma* grew very well in almost all tested pH values except 7.5 The growth of *Neurospora* was relatively slow in all pH values with pH 6.5 as the most suitable one. When the air temperature was equal to or greater than that of sawdust, less microorganisms were found in the sawdust, however, when the air temperature was lower, more microorganisms were found. In spite of pH values, more shiitake mushrooms were produced in the sawdust medium pasteurized 8 hrs at 98°C than that autoclaved 90 min. at 121°C (15 lb/in^2). The quality of shiitake mushrooms harvested from the former was also better than those form the latter. In the pasteurized medium. yields of the medium with pH values at 5 and 6 were higher than those of the medium with pH 7. Autoclaving completely killed the microorganisms in the sawdust medium while pasteurization only killed those harmful ones.

Key words : Shiitake, Cultivation, Microorganism, Production.

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INTRODUCTION

Due to the elite climate, ample source of cultivation material and appropriate technology, Taiwan is an ideal place for the production of edible mushrooms such as common mushroom (*Agaricus bisporus*) and shiitake (*Lentinus edodes*) (12). Either common mushroom or shiitake, parts of its production process such as spawn preparation, inoculation, and incubation etc. are similar. Contamination may occur at any stage of production process from spawning to havesting. Shiitake used to be grown on the logs. However, due to the shortage and increasing price of logs in the last decade, sawdust was currently used mainly for shiitake cultivation[7]. In order to reduce production cost, spawn medium was changed from wheat grain to the mixture of sawdust and rice bran. Liao[13,14] reported that *Coriolus versicolor* and *Stemonitis splendens* were the most common decaying fungi on shiitake logs. Moulds were found to be presented in the grain spawn of common mushroom, while *Alternatia, Epicoccum* and *Neurospora* spp. in sorghum spawn and *Mucor* and *Penicillum* spp. in rye spawn[2]. Harmful microorganisms, such as *Bacillus, Enterobacter, Listeria, Penicillum, Rhizopus* and *Trichiderma* in cultivation house or sawdust inhibited the mycelial growth of *Pleurotus ostreatus*[4,10,11,17]. Fruiting bodies of shiitake may also be browned by *Pseudomonas*[9]. Fungicides and/or food preservatives were used to control the contamination caused by *Aspergillus, Neurospora, Penicillium* and *Trichoderma* in the sawdust media of shiitake and *P. ostreatus*[5,8,12,15,16,18]. Contamination has been a major problem of the spawn makers and shiitake growers in Taiwan. Therefore, eliminating or reducing the cintamination problem may reduce the production cost and increase the farmers' income. The purposes of this study are : (1) to identify the contaminated microorganisms, sources of contaminants in the sawdust, shiitake spawn, inoculation and incubation rooms, and cultivation yard, (2) to explore the effects of contaminants on the mycelial growth and/or yiekd of shiitake, and (3) to evaluate the effect of medium pH values on the mycelial growth of shiitake and of the contaminants.

MATERIALS AND METHODS

Strain of shiitake and identification references : The shiitake strain used in this study was obtained from a commercial isolate which is maintained at TARI with a code No. 271. Bacteria and actionomyces were identified basing on Bergey's manual[3], while fungi were identified according to the system describede by Barnett[1].

Inspection and isolation of the contaminating microorganisms in spawn : During the spawn perparation period, visual inspection were conducted daily for one month in the spawn growth room for the percentage of contamination. The contaminated spawns were recorded and identified, if possible to the genus of the fungi or bacteria, and isolated as pure culture on potato dextrose agar (PDA), ntrient agar (beef extract 3g, peptone 5g, agar 15 g, dist. water 1 litre) and Waksman's agar (dextrose 1.0g, KH_2PO_4 0.5g, $MgSO_4 \cdot 7H_2O$ 0.2 g, $Fe_2(SO_4)$ 0.01g, egg albumen 0.25g, agar 15g, dist. water 1 litre) for further studies of identification. The total of 5,000 bottles of wheat grain and sawdust spawns were inspected from May to July and from August to Nov. 1990, respectively.

Trapping of contaminated microorganisms in the air of incubation room and cultirvation yard : Petri dishes (dis. 9 cm) containing potato dextrose agar (pH5.6) , nutrient agar (pH 7.0) or Waksman's agar (pH 8.0) were exposed to the air at the position of 1 m above the ground in the incubation room or cultivation yard for 1 hr. PDA plates were then incubated at 25°C for 96 hrs, nutrient agar (NA) at 36°C for 48 hrs and Waksman's agar at 30°C for 72 hrs for the development of colonies. Five points, 4 corners and the center of the incubation room or cultivation yard, were sampled using two plates for each point. Experiments were conducted in the incubation room at weekly intervals for five times and in the cultivation yard once every month for 7 times.

Isolation of contaminated microorganisms in the sawdust piles, cultivating sawdust bags and fruiting bodies of shiitake from cultivation yard and market : One gram each of the sawdust piled outoors and the sawdust in the cultivation plastic bags were randomly sampled at different times for isolation. After being mixed with 10 ml of distilled water, the samples were homogenized in a Waring blender as a stock solution. Isolation and identification of the microorganisms were conducted in the agar media as described previously with a series of dilution plate method[5]. Ten grams of shiitake fruiting bodies harvested from sawdust bags or purchased from the market were sampled and isolated by the same procedure. After incubating as previously described, the microorganisms on the dilution plates were identified and recorded.

Evaluation of the harmful microorganisms to the mycelial growth of shiitake : The isolated harmful microorganisms such as *Bacillus, Pseudomonas, Neurospora, Penicillium and Trichiderma*[8,9,12,15,18] were selected for testing their effect on the mycelial growth of shiitake. Ten ml each of the inoculum (1×10^8 cells/mi for bacteria, 7,500 spores/ml for fungi) were added into a sawdust bag which had which had been incoulated for each treatment. The bags were placed at three different cultivation yards in Puli, Wufeng and Mingchien for observation. One month after inoculation, the water content and pH value of the sawdust and the appearance of the bags were recorded. Normal cultivation sawdust bags without inoculation of the testing microorganisms were served as controls.

Change of pH valuses in the piled sawdusts : Sawdusts of the tree species of *Acacia confusa, Castanopsis hytrix,* and a mixture of broad-leaf trees from timber yard were piled (10x10x2.5m, W x L x H) outdoor with out covering. Sawdust pH valuses were measured once a month for 6 times. Ten grams each kind of the sawdust were sampled from the top, center and bottom of the piles with three replicates for pH measurement.

Effect of sawdust pH values on the mycelial growth of shiitake, *Trichoderma and Neurospora* : The pH values of sawdust were adjusted to 4.5, 5.0, 5.5, 6.0, 6.5, 7,0 and 7.5 with calcium carbonate powder or 300 ppm of citric acid solution. After pasteurization (98°C, 8 hrs) , 30ml of the adjusted sawdust were placed and pressed into a petri plates (diam. 9cm) for inoculation. Pure cultrues of shiitake, *Trichoderma* and *Neurospora* on PDA were used as inocula. One agar disc from each pure culture (diam. 0.5cm) was inoculated on to the center of the sawdust medium in petri dish. Sizes of the colonies were measured after incubation for 14 days at 24°C.

Evaluation of disinfection method and sawdust pH value on the degree of contamination and yield of shiitake : Sawdust medium containing water (60%) , sawdust (33.3%) , rice bran (4%) and wheat meal (2.7%) was adjusted with calcium carbontae or citric acid as previously described to obtain pH values at 5.0, 6.0 and 7.0 for testing. Polypropylene plastic bags containing 1.5Kg of the prepared medium were sterilized at 121°C, 151b/in^2, for 90min or pasteurized at 98°C for 8hrs. After cooling and before the inoculation of shiitake spawn, microorganisms in the bags were detected by the dilution method as previously described. The bags were then inoculated with shiitake spawn for the production of shiitaks fruiting bodies. The period from inoculation to the formation of fruiting bodies as well as the fresh weight of fruiting bodies per bag were recorded.

RESULT

Contaminated microorganisms in the shiitake spawns : As shown in Table 1, *Trichoderma, Penicillium, Neurospora,* and *Aspergillus* were the most frequently found microorganisms in the sawdust spawns, while *Trichoderma, Bacillus, Penicillium* and *Aspergillus* were often isolated in the wheat grain spawns. Percentage of contamination was recorded as 2.76 % or 134 bottles out of 5,000 bottles in the wheat grain spawns and 2,16% or 108 bottles in the sawdust spawns. The mycelium of shiitake grew more quickly in the wheat grain medium than that in the sawdust medium.

Table 1. Number and percentage of contaminated microorganisms in the shiitake wheat grain and sawdust

Mircroorganism	Wheat grain spawn*		Sawdust spawn	
	infected bottles	%	infected bottles	%
Alternaria sp.	2**	1.5***	1**	0.9***
Aspergillus sp.	15	11.2	10	9.3
Bacillus sp.	18	13.4	4	3.7
Bacteria unidentified	4	3.0	2	1.9
Chaetomium sp.	5	3.7	8	7.4
Fusarium sp.	5	3.7	6	5.5
Fungi unidentified	4	3.0	1	0.9
Helminthosporium sp.	11	8.2	8	7.4
Penicillium sp.	15	11.2	16	14.8
Pseudomonas sp.	11	8.2	2	1.9
Trichoderma sp.	38	28.4	34	31.5
Mucor sp.	2	1.5	4	3.7
Neurospora sp.	4	3.2	12	11.1
Total	134	100	108	100

*. Spawns were kept in the incubation room at 20°C. Days required from ionculation of shiitake inoculum to cover the whole bottles of medium were 30 days for wheat grain spawns and 35 days for sawdust spawns. Experiments for wheat grain spawns and sawdust spawns were conducted from May to July, and from August to November, 1990, respectively.

**. Accumulated number of the contaminated bottles in one month of observation from 5,000 bottles of spawns.

***. Percentage of the contaminated microorganism per se over the total number of contamination.

Contaminated microorganisms in the air of incubation room and cultivation yard : Because the incubation room is always disinfected with chemicals before use. Therefore, there was only a few number of microorganisms trapped in the air. However, the population of microorganisms increased with incubation time. Trapping efficacy varied with different media for testing. PDA was more suitable for trapping fungi, and NA for bacteria. Only a few amount of microorganisms were trapped in waksman's agar. In the cultivation yard the number of trapped microorganisms was greatly affected by the air temperature. Least number was observed in January or February which was the coldest season in Taiwan. More microorganisms were trapped in the air of cultivation yard than that in the incubation room (Table 2, 3).

Table 2. Colony number of the microorganism trapped in the air of incubation room at different times.*

Week after inoculation of shiitake	Bacteria/plate			Fungi/plate		
	PDA**	NA	WA	PDA	NA	WA
1	2***	6	2	4	0	1
2	2	14	4	18	1	1
3	4	18	5	35	3	1
4	5	20	8	40	3	2
5	6	26	8	44	4	3
Average	3.8	16.8	5.4	28.2	2.2	1.6

*. Sampling was conducted at 1 m above the ground in the incubation room (22°C) of shiitake.
**. PDA : potato dextrose agar ; NA : nutrient agar ; WA : Waksman's agar.
***. Colony number of the medium in a petri plate (diam. 9cm) exposed to the air for one hr and then incubated 96 hrs at 25°C for PDA ; 48hrs at 36°C for NA and 72 hrs at 30°C for WA. Each numger was the average of 10 plates. Colony of actinomyces was included in bacteria.

Table 3. Colony number of the microorganism trapped in the air of cultivation yard*

Month after inoculation of shiitake	Cultivation stage of stiitake	Bacteria/plate			Fungi/plate		
		PDA**	NA	WA	PDA	NA	WA
0	Inoculation	8***	28	16	30	6	8
1	Incubation	12	36	16	80	16	4
2	Incubation	11	24	10	87	12	4
3	Incubation	6	20	7	45	6	3
4	Incubation	4	22	7	32	4	3
5	Fruiting	2	9	4	21	8	2
6	Harvesting	16	43	22	115	24	10
Average		8.4	26.0	11.7	58.6	10.9	4.9

*. Sampling was conducted at 1 m above the ground in the incubation room (22°C) of shiitake.
**. PDA : potato dextrose agar ; NA : nutrient agar ; WA : Waksman's agar.
***. Colony number of the medium in a petri plate (diam. 9cm) exposed to the air for one hr and then incubated 96 hrs at 25°C for PDA ; 48hrs at 36°C for NA and 72 hrs at 30°C for WA. Each number was the average of 10 plates. Colony of actinomyces was included in bacteria.

Microorganisms in different kind of sawdusts and fruiting bodies : In general, sawdust piled outdoor for 6 months contained more microorganisms than the cultivating one and most of these microorganisms were bacteria and actinomyces. Fresh fruiting bodies right after being harvested contained the least amount of microorganisms on their surfaces. During the matketing or transportation process, population of the microorganisms on the surface of fruiting bodies increased greatly (Table 4).

Table 4. Comparison of cfu of microorganisms presented in the sawdust piled outdoor, cultivating sawdust with shiitake mycelia, and fruiting bodies from cultivation yard and/or purchased from market.

Medium	sawdust piled 6 months outdoor	Cultivating sawdust with shiitake mycelia	Fruiting bodies from cultivation yard	Frutitng bodies from marker
Potato dextrose agar	8×10^{6}*	7×10^{4}*	8×10^{5}**	6×10^{4}*
Nutrient agar	4×10^{8}	6×10^{6}	5×10^{5}	6×10^{8}
Waksman's agar	6×10^{7}	4×10^{6}	4×10^{4}	3×10^{7}
Average	1.6×10^{8}	3.4×10^{6}	3.1×10^{5}	2.1×10^{7}

*. Colony forming unit (cfu) of microorganism presented in one gram of sawdust, data were estimated with a dilution plate method, microorganism included fungi, bacteria.
**. Number of microorganism presented in 10 grams of fresh fruiting bodies harvested in the cultivation yard or purchased from market. Shiitake mycelium colonies were not included.

Comparison of the microorganisms in the normal and contaminated cultivating bags at different locations : Results indicated that less amount of microorganisms were presented in the normally grown sawdust than in the contaminated ones. The pH value of contaminated sawdust was higher than that of the normal one. No difference was observed between the water contents of these two kind of sawdusts. Among the three cultivation yards, the number of microorganism detected in sawdusts in decending order was Wufeng, Mingchien, and Puli where were 80m, 250m and 400m above the sea level respectively. This indicated that cooler place is more suitable for the growth of shiitake (Table 5). Mycelial growth of shiitake was completely inhibited in the sawdust medium inoculated or contaminated with harmful microorganisms such as *Trichoderma, Neurospora, Aspergillus, Penicillium, Bacillus* and *Pseudomonas* (Fig. 1).

Table 5. Comparison of microorganim, water content and pH values in the contaminated cultivating bags at different locations*

Location	Normal cultivating bags			Contaminated cultivating bags		
	number of microorganism per gram of sawdust (cfu/plate)	water content of sawdust (%)	pH value of sawdust	number of microorganism per gram of sawdust (cfu/plate)	water content of sawdust (%)	pH value of sawdust
Puli (400 m above sea level)	3×10^{4}	64	4.0	1×10^{9}	61	6.0
Wufeng (80 m above sea level)	4×10^{5}	63	3.8	6×10^{9}	60	6.2
Minchien (250 m above sea level)	8×10^{4}	64	4.1	3×10^{8}	61	6.2

* Data were based on the average of 20 bags one month after the inoculation of shiitake spawns.

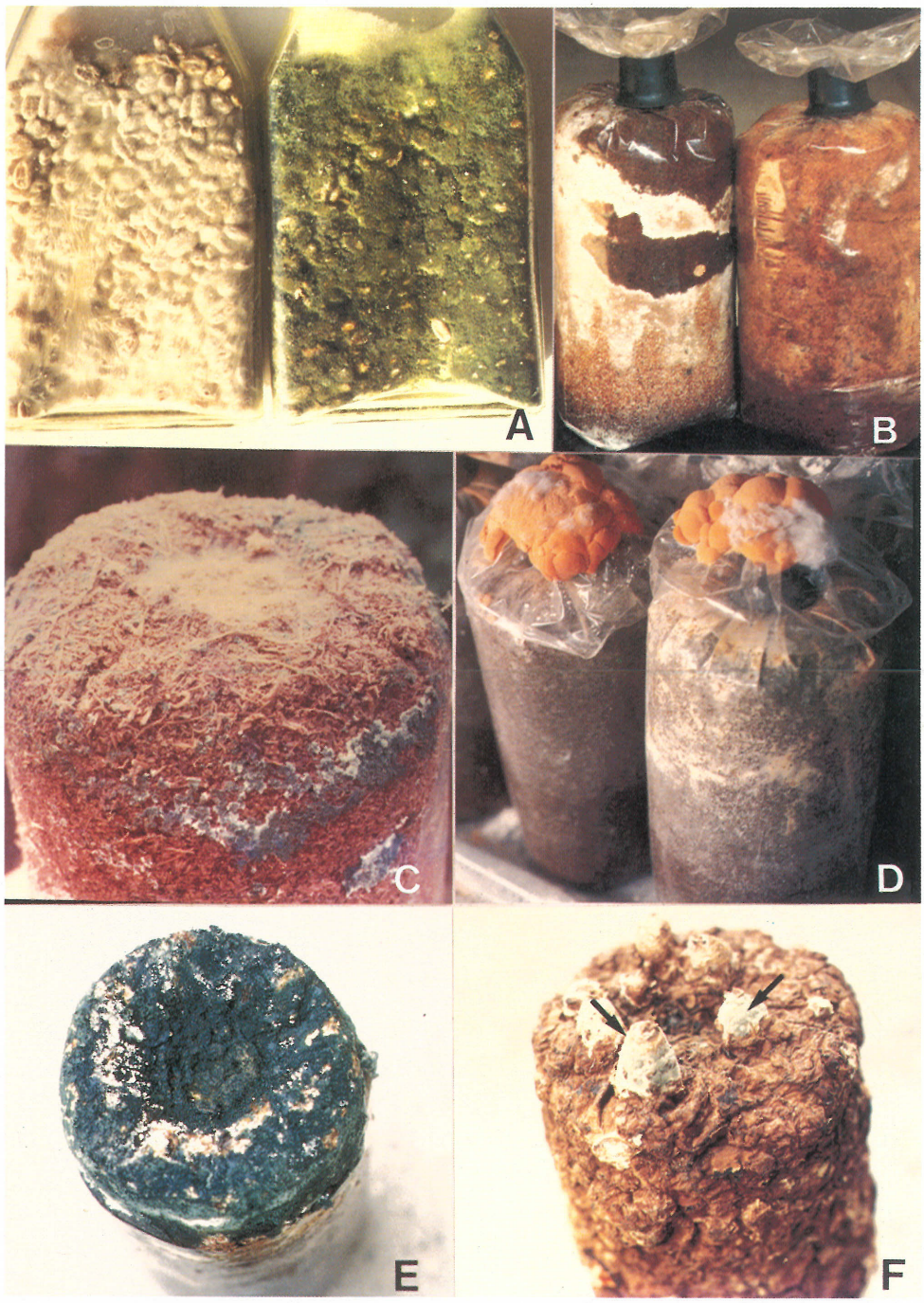

Fig. 1. Microorganisms affecting the growth of mycelium and fruiting body of shiitake : A. *Trichoderma* on wheat grain spawn (right) and healthy control (left) ; B. *Bacillus* stopped the mycelial growth (left) and *Pseudomonas* caused rotting of sawdust medium (right) ; C. *Penicillum* stopped the mycelial growth of shiitake ; D. *Neurospora* stopped the myeclial growth of shiitake ; E. *Trichoderma* stopped the mycelial growth of shiitake ; F. *Trichoderma* stopped the growth of fruiting bodies (arrow points) of shiitake.

Change of pH values in the piled sawdust : The pH value of the sawdust of *C. hystrix* was very low within the first two months of piling. It gradually increased with time and reached ca. 6.0 in 3-4 moths which is a suitable level for the preparation of sawdust medium. The pH values of *A. confusa* sawdusts and broad leaf trees also increased with time of piling. However, the change of their pH was not as evident as that of *C. hystrix* (Table 6).

Table 6. Comparison of pH value in various kind of sawdusts piled for different times*

piling time (month)	Sawdust pH value of		
	C. hystrix	A. confusa	mixed broad leaf trees
0	3.42	5.93	5.20
1	4.55	6.09	5.88
2	5.02	6.95	6.21
3	5.67	6.63	6.44
4	6.61	6.05	6.40
5	5.98	5.93	5.65
6	5.95	5.90	5.63

*. Date were based on the average of three replicaties.

Effect of sawdust pH values on the mycelial growth of shiitake, Trichoderma and Neurospora : The mycelium of shiitake grew best in the acidic conditions where the pH value was between 4.5 and 6.0. *Trichoderma* grew well in almost all tested pH values except 7.5. The growth of *Neurospora* was relatively slow in all pH values with pH 6.5 as the most suitable one (Table 7, Fig. 2).

Table 7. Effect of sawdust pH value on the mycelial growth of shiitake, Trichoderma and Neursopora

pH value	Colony size* (cm) of		
	Shiitake	Trichoderma	Neurospora
4.5	9.0	9.0	1.0
5.0	9.0	9.0	1.0
5.5	9.0	(9.0)**	1.4
6.0	8.2	(9.0)**	2.3
6.5	3.2	9.0	2.7
7.0	0.8	9.0	1.0
7.5	0.5	0.6	1.0

*. Data were the average of 3 replicates. Fungi were grown on sawdust medium 14 days at 24°C.
**. The mycelial growth of Trichoderma were relatively loose and rare.

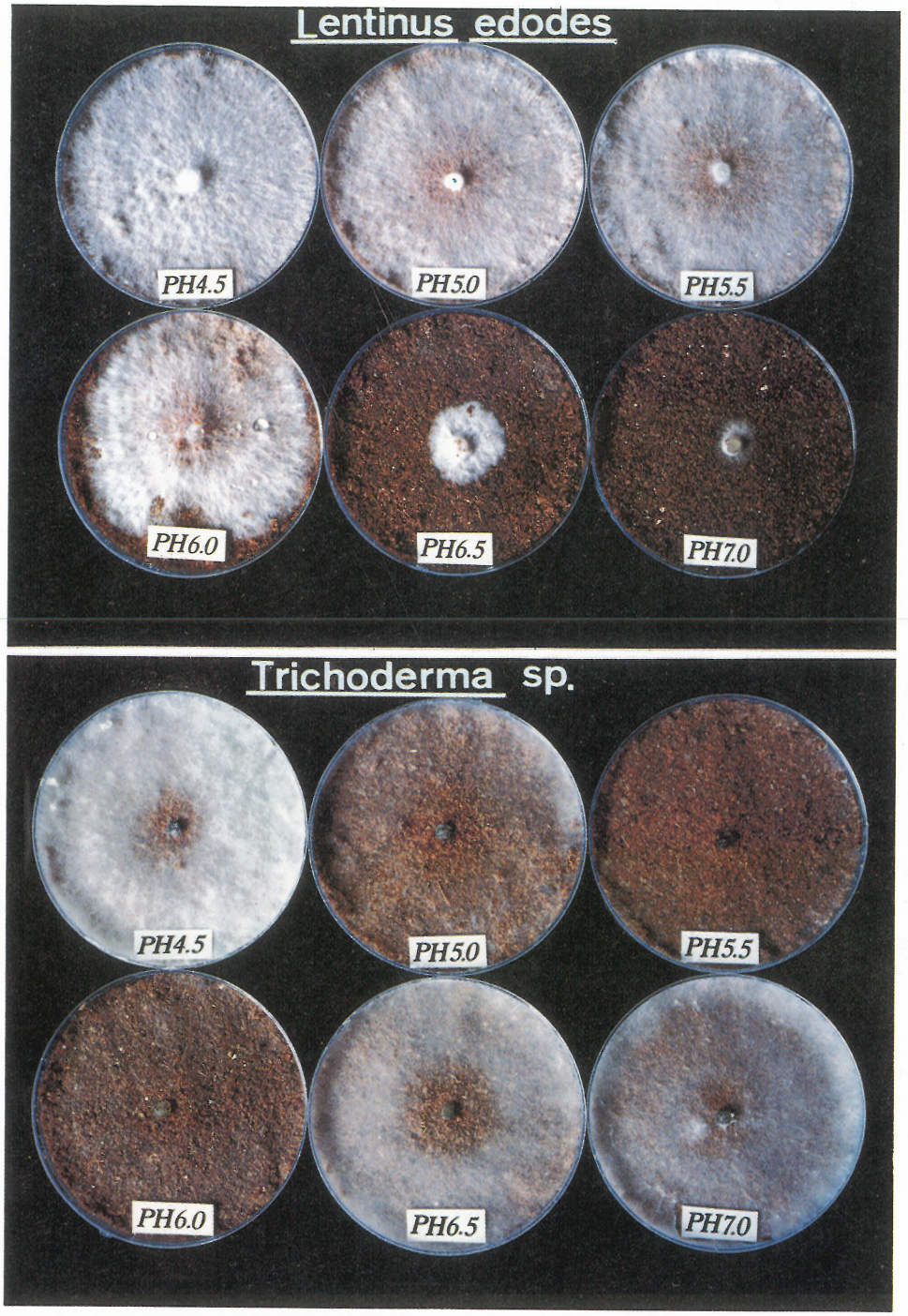

Fig. 2. Effect of pH values of sawdust medium on the mycelial growth of *Lentinus edodes* (top) and *Trichoderma* sp. (bottom).

Relationship between the air or sawdust temperature and the number of microorganisms in the sawdust at different stages of shiitake cultivation : Air and sawdust temperatures varied with the seasonal changes of temperature. When the air temperature was high in the natural condition, the tmeperature of the air and sawdust in the cultivation yard also were high. The number of microorganisms detected in the sawdust medium was coincided with the temperature difference between the sawdust and air. When the air temperature was lower than that of sawdust, more microorganisms were existed in the sawdust. Microorganism population was found to be not correlated to the cultivation stages of shiitake (Table 8).

Table 8. Relationship between number of microorganisms in the sawdust and temperatures of air and medium at different stages of shiitake cultivations*

Date	Stage of cultivation	Temperature (°C) of		Number (cfu) of microorganism ** (Per gram of sawdust)
		Air	Sawdust	
15. Sept. 1989	inoculation	24	24	4×10^3
10. Oct. 1989	middle mycelial growth	25	28	3×10^6
25. Oct. 1989	complete mycelial growth	22	21	2×10^4
15. Feb. 1989	fruiting body formation	18	19	3×10^5

*. Data were the average of 10 bags at Wufeng. Taichung (80 m above sea level).
**. cfu : colony forming unit.

Effect of disinfection method and different sawdust pH values on the contamination and yield of shiitake : In spite of pH values, more shiitake was produced in the sawdust medium disinfected or pasteurized 8 hrs at 98°C than that autoclaved 90 min at 121°C (15 lb/in^2). The quality of shiitake harvested from the pasteurized medium. However. better than that harvested from the sterilized or autoclaved medium. However, pasteurized medium took 10 -45 days more to produce fruiting bodies. In the pasteurized medium, the yields of medium with pH values at 5 and 6 were better than the medium with a pH value at 7. However, no differences were observed among the yields of autoclaved sawdust medium with different pH values. Autoclaving completely killed the microorganisms in the sawdust medium while pasteurization only eliminated the harmful ones (Table 9).

Table 9. Effect of disinfection method and pH value of sawdust on the yield and quality of stiitake as well as the number of microorganism

Disinfection method	Survey items	pH value of the sawdust		
		5.0	6.0	7.0
121°C, 15 lb/in^2 for 90 min	Fresh shiitake (g/bag) *	210a	215a	200a
	Inoculation to fruiting (days)	100	105	135
	Quality of fruiting body	large number of small fruiting bodies	large number of small fruiting bodies	large number of small fruiting bodies
	microorganism/g sawdust ** (cfu)	0	0	0

(continued)

98°C for	Fresh shiitake (g/bag) *	240b	255a	220c
8 hrs	Days from inoculation to fruiting	110	115	150
	Quality of fruiting body	large number of normal fruiting bodies	large number of normal fruiting bodies	large number of normal fruiting bodies
	microorganism/g sawdust ** (cfu)	3×10^2	4×10^2	8×10^4

*. Data within row followed by the same letters are not significantly different (p=0.01) according to D M R test. Data were the average of 200 plastic bags from 5 harvesting.
**. Number of microorganisms were detected before the inoculation of shiitake spawns.

DISCUSSION

Contamination of microorganism is a very important problem in the cultivation of edible mushrooms. This study revealed that contamination problem in the wheat grain spawns is greater than that in the sawdust spawns. This is possibly because the wheat grains provides more nutrients for the growth of microorganism especially bacteria than the sawdust. Since spawn cost is becoming more popular in the last decades.

Although Binter(2) reported that the important pathogens, such as *Mycogone* and *Diehliomyces,* were not presented in the spawn of common mushroom, this research indicated that the important pathogen of shiitake production in sawdust bags such as *Trichoderma,* was presented in the spawns and also in the air of the incubation room and cultivation yard (Table 2,3). Therefore, in order to reduce the percentage of contamination during the preparation process of spawns, establishment of a laminar flow filter system in the inoculation and incubation rooms is necessary. Good pasteurilization of the sawdust is also very important which may reduce the sources of primary inoculum. Good management of the environments in the incubation room and cultivtion yard may reduce the contamination of cultivation bags. The amount of microorganisms on the surfaces of fruiting bodies (Table 4) during marketing or transportation process may be reduced by precooling or low temperature storage. Cultivation trials at three locations showed that higher altitude areas are more suitable for the growth of shiitake than in the low altitude areas (Table 5). This is possibly because the low temperature favored the mycelial growth and fruiting of shiitake and the growth of contaminating microorganisms were reduced.

The change of pH values in the sawdust of different tree species piled for different time of piling (Table 6) showed piling not only changed the pH value but also changed the microflora in the sawdust. Different fungi have their own suitable optimal pH values for their growth (Table 7). Population change of the microorganisms in different stages of cultivation indicated that not only the temperature of the air the sawdust but also the biological activity influenced the population density of the microorganisms. In the early stage, because sawdust was just pasteurized or autoclaved, the population of harmful microorganism was very low in it. However, after inoculation of shiitake spawn, the population increased with time. The biological heat released by shiitake mycelium also favored the growth of harmful or contaminated microorganisms (Table 8). Disinfection experiments indicated that pasteurization at 98°C for 8 hrs was better than sterilization (Table 9)

because sterilization eliminates all the microorganisms in the medium, including those beneficial ones.

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香菇栽培之污染微生物及其對香菇生產之影響[1]

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摘　要

香菇污染雜菌一直是香菇栽培者及菌種製造者之主要問題，而減少污染程度則是降低成本及增加收益的方法。本文旨在檢測存在於菌種、木屑及培養基或栽培場、培養室等之微生物，及這些微生物對香菇生產之影響，進而尋求適當之 pH 值與殺菌方式以便降低微生物之污染藉以改進香菇栽培技術。由試驗結果可知，在香菇木屑菌種中常見之雜菌以 *Trichoderma, Penicillium, Neurospora* 及 *Aspergillus*最多，而在麥粒菌種中則以 *Trichoderma, Bacillus* 及 *Aspergillus* 爲主。香菇菌絲在麥粒菌種中之生長速度比在木屑菌種中爲快。在栽培場所收集之微生物數量比在接種室或培養室者爲多，而堆積在戶外之木屑其微生物含量又比在木屑培養基中者爲多。在運輸販賣過程中香菇子實體表面會污染許多微生物。將這些有害微生物接種於木屑包，則可發現正常生長之木屑包其微生物量較污染包爲低且 pH 值也較低，含水量則正常包比污染包稍高，但差異不大。木屑在戶外堆積六個月後可發現火燒柯（*Castanopsis hytrix*）由原來的低 pH 值漸漸昇高爲適合香菇生長的範圍，而相思樹（*Acacia confusa*）及闊葉樹混合木屑也有相同的結果，惟其變化不如火燒柯大。酸性木屑培養基極適合香菇菌絲之生長，較適合之 pH 值爲4.5至6.0，*Trichoderma* 則除了pH7.5外，所有供試之範圍均適合生長，而 *Neurospora* 則在所有測試 pH 值中均生長緩慢，只有 pH6.5較適合。當氣溫高於或等於木屑包溫時，則微生物量較少，反之木屑包溫高於氣溫時，則微生量較多。不論木屑包在何種 pH 值，若以98°C，8小時殺菌之處理時，香菇的產量或品質均優於用121°C（15lb/in²），90分之殺菌方式。後者之殺菌方式除了可殺滅害菌外，其它有益微生物也一併殺死，而 pH 5 及 pH 6 則在產量及菇質方面均優於 pH 7。

關鍵詞：香菇、栽培、微生物、生產。

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